CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER: 021083

PHARMACOLOGY REVIEW(S)

NDA 21-083
Date Submitted: 12/15/1998
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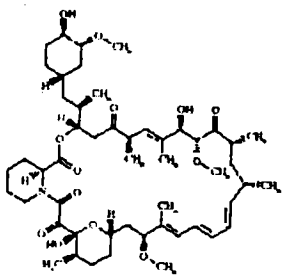
Related NDAs: None
Related INDs: [redacted]
HFD-590

Sponsor: Wyeth-Ayerst

Drug: Rapamune™
(3*S*,6*R*,7*E*,9*R*,10*R*,12*R*,14*S*,15*E*,17*E*,19*E*,21*S*,23*S*,26*R*,27*R*,34a*S*)-9,10,12,13,14,21,22,23,24,25,26,27,32,33,34,34a-hexadecahydro-9,27-dihydroxy-3-[(1*R*)-2-[(1*S*,3*R*,4*R*)-4-hydroxy-3-methoxycyclohexyl]-1-methylethyl]-10,21-dimethoxy-6,8,12,14,20,26-hexamethyl-23,27-epoxy-3*H*-pyrido[2,1-*c*][1,4] oxaazacyclohentriacontine-1,5,11,28,29 (4*H*,6*H*,31*H*)-pentone.



Formulation: Each mL of Rapamune Oral Solution contains 1 mg sirolimus; inactive ingredients are Phosal 50 PG (phosphatidylcholine, propylene glycol, monodiglycerides, ethanol, and soy fatty acids) and Polysorbate 80, NF.

INTRODUCTION

Rapamycin is an immunosuppressant macrolide, structurally related to FK-506 (Tacrolimus™), which was developed for organ transplantation.. Rapamycin was isolated from *Streptomyces hygroscopicus*. Rapamycin appears to have a unique immunosuppressive biochemical mechanism of action, distinct from that of cyclosporin (CsA), tacrolimus (FK506), mycophenolate mofetil, or azathioprine. Rapamycin inhibits T lymphocyte activation and proliferation that occurs in response to antigenic and cytokine (Interleukin [IL]-2, IL-4, and IL-15) stimulation by a mechanism different from that of other immunosuppressants. In cells, rapamycin binds to the immunophilin, FK binding protein 12 (FKBP-12), producing an immunosuppressive complex. Unlike cyclosporin and tacrolimus, the rapamycin FKBP complex appears to have no effect on calcineurin activity. This complex binds to and inhibits the activation of a kinase called the mammalian target of rapamycin (mTOR). Inhibition of mTOR by rapamycin suppresses cytokine-driven T-cell proliferation, inhibiting the progression from the G1 to the S phase of the cell cycle.

Rapamycin prolongs allograft survival in animal models of transplantation, including rodents and primates, both for solid organ and for cellular allografts. Nephrotoxicity is a concern with many preceding immunosuppressants, particularly cyclosporin. Rapamycin, perhaps due to its differing mechanism of action, appears not to have the nephrotoxicity seen with cyclosporin. Combinations of rapamycin with other immunosuppressants remain problematic. Adverse effects seen with other immunosuppressive drugs, including elevation of glucose and hyperlipidemia, are of concern with rapamycin also.

All studies performed at Wyeth-Ayerst Research, Chazy, NY and glp unless otherwise noted. Numerous manuscripts were included by the sponsor and noted as non glp studies.

PHARMACOLOGY STUDIES

Neuropharmacology

CNS Evaluation of Rapamycin and Cyclosporin-A in Global Behavioral Assessment and Spontaneous Locomotor Activity Tests GTR-18509

Cardiovascular/Respiratory Pharmacology Effects of Acute Oral Administration of Rapamycin and Cyclosporin-A on the Mean Arterial Blood Pressure and Heart Rate in Spontaneously Hypertensive Rats GTR-18809

Calcineurin As a Common Target Mediating Cyclosporin-A and FK506 Induced Sympathetic Neural Activation GTR-22205 (attached)

Effect of Rapamycin on Isolated Rat Thoracic Aorta: An *in vitro* Study GTR-22339

Effects of Rapamycin and Cyclosporin on Pulmonary Functions in the Anesthetized Guinea Pig GTR-18895

Gastrointestinal Pharmacology

Effects of Rapamycin on Gastric Acid Secretion and Emptying and on the Morphology Integrity of the Gastrointestinal Mucosa GTR-18897

Renal Pharmacology

Mouse

Effect of Rapamycin on Kidney Function in the Mouse GTR-20545 (attached)

Rat

Effects of Rapamycin on Renal Function in Conscious Saline-Loaded Rats GTR-18816

Kidney Function in the Adjuvant Arthritic Rat; Effects of the Immunosuppressive Macrolides Rapamycin and Cyclosporin-A GTR-20113

Effect of Rapamycin on Kidney Function in the Female Spontaneously Hypertensive Rat GTR-20815

Effect of Rapamycin on Kidney Function in the Sprague-Dawley Rat GTR-18900

Renal Effects of Rapamycin in the Spontaneously Hypertensive Rat GTR-21287 (attached)

Nephrotoxicity of Immunosuppressants in Rats: Comparison of Macrolides with Cyclosporin GTR-23817

Musculoskeletal Pharmacology Rapamycin: A Bone Sparing Immunosuppressant? GTR-24187

The Effect of Low Dose Combined Immunosuppressants Cyclosporin-A and Rapamycin on Bone Metabolism and the Endocrine System of Adult Male Rats GTR-31267

Endocrine Pharmacology

Acute Effect of Rapamycin (AY-22989) on Plasma Glucose in the Rat GTR-18898

Analgesic Pharmacology

Effect of Rapamycin on the PBQ-Induced Writhing Response in Mice GTR-19072

Reproductive Pharmacology

Reproductive Evaluation of AY-22989 and WY-46448 in Adult Male and Female Rats GTR-18905

Other *In Vitro* Studies
Comparison of the Effect of Rapamycin and FK506 on Release of Prostacyclin and Endothelin In Vitro GTR-21842 (attached)

Rapamycin and FK-506 do not Affect Platelet Aggregation of Mitochondrial Function GTR-20654

Effect of Rapamycin on Receptor Binding in Nova Screen Profile[TM] GTR-18817

PHARMACOLOGY STUDY REVIEWS

CNS evaluation of rapamycin and cyclosporin-A in global behavioral assessment and spontaneous locomotor activity tests
GTR-18509 non glp
16 July 1990

Male Sprague Dawley rats were treated with either rapamycin (0.5 0r 2.5 mg/kg), cyclosporin (5 or 25 mg/kg), or vehicle by a single i.p. injection. Global activity and locomotor activity were monitored for 60 minutes following treatment. Small reductions in spontaneous locomotor activity were seen at the low doses of both drugs; no other overt behavioral signs were seen.

Effects of acute oral administration of rapamycin and cyclosporin-A on the mean arterial blood pressure and the heart rate in spontaneously hypertensive rats
GTR-18809 non glp
14 August 1990

Spontaneously hypertensive rats were treated with a single oral dose of either rapamycin (3 mg/kg) or cyclosporin (15 mg/kg) and cannulated. Neither cyclosporin nor rapamycin had any effect on either mean arterial blood pressure or heart rate.

Effect of rapamycin on isolated rat thoracic aorta: an *in vitro* study
GTR-22339 non glp manuscript
11 November 1993

Thoracic aortas from Sprague Dawley rats were prepared as intact and denuded rings for in vitro administration of rapamycin (10, 100 and 1000 ng/ml) or polyethylene glycol vehicle control. Responses to the thromboxane A2 analog U46619 and phenylephrine to the treated rings were measured. The vehicle induced tension which rapamycin antagonized in intact rings. This was not seen in denuded rings. Rapamycin had no effect on contraction caused by U46619 or phenylephrine in intact, and to U46619 in denuded rings.

Effects of rapamycin and cyclosporin on pulmonary functions in the anesthetized guinea pig.
GTR-18895 non glp
10 August 1990

Rapamycin (3 mg/kg i.p.), cyclosporin (15 mg/kg i.p.) or vehicle were administered to vagotomized guinea pigs in a single dose. Pulmonary resistance and compliance and heart rate and blood pressure were determined. No significant effects on pulmonary or cardiovascular parameters resulted from either rapamycin or cyclosporin.

Effects of rapamycin on gastric acid secretion and emptying and on the morphologic integrity of the gastrointestinal mucosa
GTR-18897 non glp
18 October 1990

Rapamycin was given to male Sprague Dawley rats by oral gavage (3 mg/kg) either as a single dose or daily for three days. Cyclosporin was given by oral gavage (15 mg/kg) on the same schedules. Indomethacin was used as a positive control for gastric irritation. Gastric acid secretion was antagonized by cyclosporin (atropine positive control) but not rapamycin and gastric emptying was delayed by cyclosporin but not rapamycin. Neither drug had any effect on the morphologic integrity of either the gastric or intestinal mucosa.

Effects of rapamycin on renal function in conscious saline-loaded rats
GTR-18816 non glp manuscript

Male Sprague-Dawley rats were given rapamycin (1 or 3 mg/kg), cyclosporin (5 or 15 mg/kg) or vehicle (4% DNA, 3% polysorbate 80 and 50% PED-300) by gastric gavage in a single dose. Urine was collected for analysis. Neither drug had any effect on urine flow rate, sodium or potassium excretion, or urine osmolality. A slight pH decrease was seen in the 3 mg/kg rapamycin rats

Kidney function in the adjuvant arthritic rat; effects of the immunosuppressive macrolides rapamycin and cyclosporin-A
GTR-20113 non glp manuscript
25 September 1991

In two studies, Lewis rats, arthritis induced with heat-killed M. butyricum injected into the hind foot pad, were treated with rapamycin (10 mg/kg), cyclosporin (10 mg/kg) , cyclophosphamide (10 mg/kg) or vehicle (0.5% Tween 80). A normal rat control was included. In a second study, the groups consisted of vehicle, rapamycin (10 mg/kg) or cyclosporin (10 mg/kg) and normal rats. In both studies animals were dosed three times per week for two weeks with two weeks recovery. Urine was collected on days 16 and 29. By day 16 renal dysfunction of the adjuvant treated rats was seen (increased urine volume and decreased creatinine clearance, increased paw volume) and was decreased by all of the drug treatments. At day 30, only rapamycin and cyclophosphamide treated rats continued to show improved renal function in the adjuvant treatment model. Histopathologic effects of the kidney were seen at day 16 in both cyclosporin and cyclophosphamide treated rats (tubule granular deposits and glomerulopathy) but not in rapamycin treated rats.

Effects of rapamycin on kidney function in the female spontaneous hypertensive rat
GTR-20815 non glp manuscript
30 July 1996

Female spontaneously hypertensive rats (Sprague-Dawley [redacted] were treated with rapamycin (10 mg/kg), cyclosporin (35 mg/kg) or control (4% cremaphor, 2% alcohol) by oral gavage for 14 days. Urine was collected on day 14. Rapamycin did not affect renal parameters (urine output, plasma creatinine, creatinine clearance or kidney weight) while cyclosporin decreased plasma creatinine clearance and increased plasma creatinine. Histopathology signs included necrotizing vasculotitis (3/7, rapamycin) and inclusion bodies, juxtaglomerular hypertrophy, tubular vascularization and necrotizing vasculopathy (all cyclosporin treated rats) .

Effect of rapamune on kidney function in the Sprague-Dawley rat
GTR-18900 non glp manuscript
6 November 1995

Male spontaneously hypertensive rats (Sprague-Dawley [redacted] studies were implanted with osmotic pumps delivering either vehicle (controls; cremaphor and alcohol), rapamycin (0.01, 0.02, 0.04, 0.08, 0.8 mg/kg), cyclosporin (2 mg/kg) or 2.0 mg/kg rapamycin i.v, for 14 days, followed by urine collection in metabolic cages for 14 days. At terminal necropsy rats receiving at least 0.01 mg/kg rapamycin demonstrated necrotizing vasculopathy and decreased creatinine clearance. The clearance was restored following recovery. Tubular atrophy and inclusion bodies of the proximal tubules were seen at doses of 0.02 mg/kg rapamycin and above, in a dose related response.

Nephrotoxicity of immunosuppressants in rats: comparison of macrolides with cyclosporin
GTR-23817 non glp manuscript
31 March 1998

FK506 (5 mg/kg, s.c.), cyclosporin (50 mg/kg, s.c.) and rapamycin (5 mg/kg, s.c.) were compared for nephrotoxicity in Wistar rats for 10 days. FK506 and cyclosporin induced reduced creatinine clearance, hypomagnesemia and hyperuricemia. FK506 and cyclosporin also produced effects in proximal tubular epithelium including atrophy, vacuolization, inclusion bodies, microcalcification , focal mononuclear infiltrate and hypertrophy of the juxtaglomerular apparatus. None of these effects were seen in the rapamycin treated rats.

Rapamycin: a bone sparing immunosuppressant?
GTR-24187 non glp manuscript
9 March 1994

Sprague Dawley rats were treated with vehicle, cyclosporin (15 mg/kg), FK 506 (5 mg/kg) or rapamycin (2.5 mg/kg) by oral gavage for 28 days. Bone parameters were compared at necropsy. On days 0, 14 and 28, blood was collected for hormone analysis. Bone turnover, osteoporosis and elevated serum vitamin D and osteocalcin were seen in cyclosporin and FK 506 treated rats. Trabecular bone area was decreased, weight gain was decreased and hyperglycemia developed also. Rapamycin treated rats had increased bone remodeling

The effect of low dose combined immunosuppressants cyclosporin-A and rapamycin on bone metabolism and the endocrine system of adult male rats.
GTR-31267 non glp manuscript
16 January 1998

Male Sprague Dawley rats received either vehicle, cyclosporin (1.5 or 7.5 mg/kg), rapamycin (0.25 mg/kg) or cyclosporin (1.5 mg/kg)+ rapamycin (0.25 mg/kg) for 3 months. Blood was collected at 0, 1, 2 and 3 months of treatment for endocrine hormone determination. Serum osteocalcin was increased in both cyclosporin groups at 2 months, while rapamycin decreased levels at 3 months. Cyclosporin also increased IGF-1 a 2 months, while rapamycin increased it at 2 and 3 months. Combination treatment did not affect IGF-1. Cyclosporin also reduced trabecular bone area and number.

Acute effect of rapamycin on plasma glucose in the rat
GTR-18898 non glp manuscript
5 September 1990

Male Sprague Dawley rats received vehicle, rapamycin (0.5 mg/kg), cyclosporin (15 mg/kg) or tolbutamide (50 mg/kg) in a single oral dose. Blood was collected at 1, 2 and 4 hr after treatment after both feeding and fasting. Neither rapamycin nor cyclosporin had any effect on blood glucose after a single dose.

Effect of rapamycin on the PBQ-induced writhing response in mice
GTR-19072 non glp
20 October 1990

CD-1 mice [redacted] were given paraphenylbenzoquinone (PBQ, 0.3 mg/kg i.p.) to induce writhing. Indomethacin (6 mg/kg, p.o.) served as positive analgesic control. Rapamycin (5 mg/kg, p.o., one hr after PBQ) did not significantly decrease writhing.

Reproductive evaluation of AY-22,989 and Wy-46,448 (cyclosporin) in adult male and female rats
GTR-18905 non glp
12 December 1990

Female Sprague Dawley rats were treated with either rapamycin (3 mg/kg, p.o.), cyclosporin (15 mg/kg, p.o.) or control (ethanol-cremaphor, p.o.) on the day of proestrus and sacrificed the following day to examine for ova. Other females were mated and treated as above on days 1 to 7 or 7 to 12 of pregnancy. Other males were treated as described above for 8 days for hormonal and reproductive tract study. Ovulation in females was not affected by either drug. No effect was seen in the rats treated on days 1-7; however, the rats treated on days 7-12 had pregnancy terminated by rapamycin. Male rats had decreased body weight in both treatment groups. Rapamycin decreased the weights of the prostate, seminal vesicles, epididymides, levator ani and thyroid. Hormone levels (FSH, LH, prolactin, testosterone, corticosterone) were unaffected.

Rapamycin and FK506 do not affect platelet aggregation or mitochondrial function
GTR-20654 non glp manuscript
16 March 1992

Human platelets and rat liver mitochondria were isolated. Platelets were preincubated with up to 100 nM rapamycin or FK 506 prior to calcium dependent or independent aggregation. Mitochondria were incubated with rapamycin, cyclosporin A, cyclosporin G, cyclosporin H, or FK506 at concentrations up to 4 μM with calcium to induce swelling. In the platelet assay , no drug effects were seen. In the mitochondrial assay, only cyclosporin A and G inhibited calcium-induced mitochondrial swelling.

Effect of rapamycin on receptor binding in Nova screen profile
GTR-18817 non glp
12 October 1990

Rapamycin was screened in the NovaScreen Profile receptor binding assay and showed no activity in sympathetic and parasympathetic, adrenergic and amino acid receptor assays, calcium channels, opiate and prostanoid receptors, adenylate cyclase or protein kinase c. Histamine was inhibited between 100 and 500 nM.

TOXICOLOGY STUDIES

Acute Toxicity Studies
Mouse
Oral
AY-22,989 Rapamycin: Acute Oral Toxicity Study - Mouse GTR-19223

Intravenous
AY-22,989 Rapamycin: Acute Intravenous Toxicity Study - Mouse GTR-19224

Rat
Oral
AY-22,989 Rapamycin: Acute Oral Toxicity Study -Rat GTR-19215

Intravenous
AY-22,989 Rapamycin: Acute Intravenous Toxicity Study - Rat GTR-19225

Multiple Dose Toxicity (Subchronic, Chronic, Carcinogenicity)
Repeated Dose Toxicity Studies
Rat
Oral
AY-22,989 (Rapamycin): Multiple Oral (Gavage) Dose Range Finding Study – Rat GTR-21204 (attached)

AY-22,989: Rapamycin Multiple Oral (Gavage) Dose Range Finding Study – Rat GTR-19209 (attached)

AY-22989 (Rapamycin): One Month Oral (Gavage) Toxicity Study - Rat GTR-20617 (attached)

Rapamycin: One Month Oral (Gavage) Toxicity Study (New Formulation) - Rat GTR-22356 (attached)

AY-22,989: Rapamycin Three Month Oral (Gavage) Toxicity Study with Recovery - Rat GTR-19371 (attached)

Rapamycin: Six Month Oral (Gavage) Toxicity Study with Recovery - Rat GTR-22821 (attached)

Rapamune: Fifty-Two Week Oral (Gavage) Toxicity Study in Rats GTR-25628 (attached)

Intravenous
AY-22,989 Rapamycin: Intravenous Dose Range Finding Study - Rat. GTR-19372

AY-22,989 Rapamycin One Month Intravenous Toxicity Study - Rat GTR-19361

Sirolimus: Twenty-Eight Day Intravenous Toxicity Study in Rats (New Formulation) GTR-23719 (attached)

Sirolimus: Twenty-Eight Day IV Tox. Study in Rats (New Formulation) (Addendum I) GTR-24931 (attached)

Dog
Oral
AY-22,989 Rapamycin: Short-Term Oral Toxicity Study - Dog GTR-T-74-26

Sirolimus: Single Dose Intravenous and Multiple Dose Oral (Gavage) Range Finding Study – Dog GTR-21939 (attached)

Rapamycin: Special One Month Oral (Gavage) Toxicity Study - Dog. GTR-22827 (attached)

Intravenous
AY-22,989: Rapamycin Single and Multiple Intravenous Dose Range Finding Study - Dog. GTR-19208

Intravaginal
AY-22,989: Rapamycin Twenty-Eight Day Intravaginal Toxicity Study - Dog GTR-T-75-9

AY-22,989: Rapamycin Four Week Vaginal Toxicity Study - Dogs GTR-T-75-30

Monkey
Oral
AY-22,989 Preliminary Tolerance Toxicity Study -Cynomolgus Monkey GTR-17270

AY-22,989 Rapamycin Multiple Oral (Gavage) Dose Range Finding Study - Monkey GTR-19213 (attached)

AY-22,989 One Month Oral (Gavage) Toxicity Study- Cynomolgus Monkey GTR-17195

AY-22,989 (Rapamycin): One Month Oral (Gavage) Toxicity Study - Monkey GTR-20618 (attached)

Rapamycin: One Month Oral (Gavage) Toxicity Study (New Formulation) - Monkey GTR-22353 (attached)

AY-22,989: Rapamycin Three Month Oral (Gavage) Toxicity Study with One Month Interim Sacrifice - Monkey GTR-19543 (attached)

Rapamycin: Six Month Oral (Gav) Toxicity Study with Recovery - Monkey GTR-21942 (attached)

Intravenous
AY-22,989 Rapamycin Intravenous Dose Range Finding Study - Monkey GTR-19212

Sirolimus: Four Week Intravenous Toxicity Study in Monkeys GTR-24394 (attached)

AY-22,989 Rapamycin: One Month Intravenous Toxicity Study - Monkey (1) GTR-20212

AY-22,989 Rapamycin One Month Intravenous Toxicity Study - Monkey GTR-19211
Sirolimus: 28 Day IV Tox. Study in Monkeys (New Formulation) (Addendum I) GTR-24907 (attached)

Range-Finding/Carcinogenicity Studies
Mouse
Oral
Sirolimus: Three Week Oral (Gavage) Dose Ranging Study in Mice (Addendum I) GTR-24957

Sirolimus: Three Week Oral (Gavage) Dose Ranging Study in Mice GTR-24429 (attached)

Rapamune Thirteen Week Oral (Gavage) Toxicity Study in Mice GTR-25497 (attached)

Rapamune: Thirteen Week Oral (Gavage) Toxicity Study in Mice (Addendum I)
GTR-26187 (attached)

Rapamune: Thirteen Week Oral (Gavage) Ranging Study in B6C3F1 Mice GTR-30332

Rapamune: A Twenty-Nine Week Chronic (Male) and Eighty-Six Week Carcinogenicity (Female) Oral (Gavage) Study in Mice GTR-32267 (attached)

Rat
Oral
Two Year Oral (Gavage) Carcinogenicity Study in Rats GTR-32266 (attached)

Special Toxicity Studies
Rat
Testicular/Bone Effects
Rapamune: Special Oral (Gavage) Study to Evaluate the Reversibility of the Effects on Testes and Bone in Young Male Rats GTR-26739

Rapamune: Special Oral (Gavage) Study to Evaluate the Effects on Bone Quality and Testes in Older Male Rats GTR-26105

Sirolimus: Multiple (62 Days) Dose Intravenous Study to Evaluate New Formulation in Rats GTR-23838 (attached)

Testosterone/Bone Effects
Rapamune: Mechanistic Study to Evaluate the Effects of Testosterone Replacement on Bone in Rapamune Treated Male Rats GTR-31388

Bone Effects
Sirolimus: Special Study to Evaluate Bone Integrity in Rats GTR-24955 (attached)

Phospholipidosis 63 1
Rapamune: 13 Week Oral (Gavage) Phospholipidosis Study in Male Rats With a 4 Week Recovery GTR-32615

Interaction
AY-22,989 (Rapamycin)/Cyclosporine/Prednisone: Multiple Dose R.F. Study - Rat GTR-20227 (attached)

AY-22,989 (Rapamycin)/Cyclosporine/Prednisone: One Month Toxicity Study - Rat GTR-20222

Toxicity of Rapamycin - A Comparative and Combination Study with Cyclosporine at Immunotherapeutic Dosage in the Rat GTR-20619

Rapamycin/Cyclosporine/Azathioprine Sodium: One Mo. Tox. Study - Rat GTR-25329

Rapamune/Cyclosporine: Thirteen Week Oral (Gavage) Toxicity Study in Rats GTR-31710

Impurity/Degradation
Rapamune and WAY-124854 (Rapamune Impurity):Twenty-Eight Day Oral (Gavage) Toxicity Study in Male Rats GTR-27324 (attached)

Rapamune and WAY-126792 (Seco-rapamycin):Twenty-Eight Day Oral (Gavage) Toxicity Study in Male Rats GTR-27581 (attached)

Rabbit
Rapamycin: Seven Day Intravenous Irritation Study in Rabbits GTR-23138
Sirolimus: DOT Acute Dermal Irritation Study on Rabbits GTR-25355

TOXICOLOGY STUDY REVIEWS

AY-22989 rapamycin: acute oral toxicity study- mouse
GTR-19223
20 December 1990

Male and female CD-1 mice [redacted] 5/sex/group, were given a single dose of rapamycin at 0 (20% DMA, 75% centrophil W, 5% polysorbate 80), 0 (twice volume control), 500 or 800 mg/kg by oral gavage. Mice were monitored for 2 weeks. During the study, deaths occurred in the double volume control (2 males, 1 female) and 1 male in the 500 mg/kg group, day 2. Drug effects seen included ptosis, rough coat and reduced motor activity (500 mg/kg males). A NOEL was not demonstrated in this study.

AY-22989 rapamycin: acute intravenous toxicity study- mouse
GTR-19224
19 December 1990

Male and female CD-1 mice [redacted] 5/sex/group, were given a single i.v. dose of rapamycin at 0 (30% DMA, 60% polyethylene glycol 400, 10% polysorbate 80), 40, 0 (50% DMA, 40% polyethylene glycol 400, 10% polysorbate 80), 150 or 250 mg/kg. Mice were observed for 14 days. During the study, 1 female (250 mg/kg) died 4 hr after dosing. Drug effects seen included ptosis, low posture for 4 hr following dosing. A NOEL was not demonstrated in this study.

AY-22989 rapamycin: acute oral toxicity study- rat
GTR-19215
20 December 1990

Male and female rats [redacted] 5/sex/group, were given a single oral dose of rapamycin at 0 (20% DMA, 75% centrophil w, 5% polysorbate 80), 0 (twice volume control), 500 or 800 mg/kg. Rats were observed for 14 days. All rats survived through the study. Effects observed on study included (500 mg/kg) ptosis, decreased motor activity, red pigmentation at eyes and mouth; (800 mg/kg) red pigmentation of eyes and mouth, and rough coat. These effects were also seen in the twice volume control and were attributed to DMA toxicity.

AY-22989 rapamycin: acute intravenous toxicity study- rat
GTR-19225
19 December 1990

Charles River CD rats, 5/sex/group, were given a single i.v. injection of vehicle (50% DMA, 40% polyethylene glycol 400, 10% polysorbate 80) or 250 mg/kg rapamycin. Rats were observed for 14 days. During the study, 2 males and 1 female in the treated group died (in the initial 4 hr). Drug effects included immobility, ataxia, tachypnea, decreased motor activity and discoloration of tail. A NOEL was not achieved in this study.

AY-22,989 rapamycin: intravenous dose range finding study- rat
Study no. GTR-19372 non glp
2 Jan 1991

Rapamycin was administered by tail vein injection to Charles River rats for seven to 28 days at doses of 0 (vehicle, PEG300, water, polysorbate 80), 0.01, 0.02, 0.03, 0.5 (seven days of treatment); 0, 0.05, 0.1, 0.25, 0.5, 1.0 mg/kg, 1-3/sex/group. Other vehicles were also examined: PEG300 + polysorbate 80, PEG300 + polysorbate 80 + diethylacetamide, water + polysorbate 80, polysorbate 80 + water + diethylacetamide and PEG300 + polysorbate 80 + water + dextrose. All rats survived the study. Injection site lesions occurred with all vehicles. The vehicle PEG300 + polysorbate 80 produced focal necrosis of the dermis and epidermis at the injection site.

AY-22,989 rapamycin: one month intravenous toxicity study-rat
Study no. GTR-19361 glp
13 Feb 1991

Rapamycin was administered by tail vein injection to Charles River rats (20 rats/sex/group) for 28 days at doses of 0, 0.025, 0.75 or 1.5 mg/kg. Survival, body weight, food consumption, physical examination, opthamology, hematology and clinical chemistry were assessed during the study. A battery of tissues were examined for histopathology at necropsy. All rats survived through the study. Body weight gain and food consumption were decreased in the 0.75 and 1.5 mg/kg groups. Hematologic changes were found in 0.025 mg/kg males (increased hematocrit, hemoglobin, rbc, lymphoctes) and 0.75 and 1.5 mg/kg (decreased mean cell volume, reticulocytes, platelets and lymphocytes) Clinical chemistry effects occurring mainly in the 0.5 and 1.5 mg/kg groups included slightly decreased ALT, alkaline phosphatase, tissue plasminogen, albumin, total and indirect bilirubin, inorganic phosphorus and creatine kinase. Creatinine, BUN, and uric acid were increased only in the first week. Glucose was increased in the 0.75 and 1.5 mg/kg group males, increasing through the study. Pancreatic vacuolation also appeared in these rats. Slight increases in liver and kidney weights occurred in drug-treated groups. Testicular and seminal vesicle atrophy and testicular giant cells were seen in 0.75 and 1.5 mg/kg group males. Increases of pulmonary macrophages were seen in the 0.75 and 1.5 mg/kg groups. The 0.025 mg/kg dose appeared to be the NOAEL in this study

AY-22,989 rapamycin: short term oral toxicity study –dog
Study no. GTR-T-74-26 non glp
7 Nov 1974

Rapamycin was given orally to one male and one female beagle dog, 200 mg/kg, for 5 days, followed by a 10 day observation period. During the study the male had emesis and diarrhea, anorexia, body weight loss, red lesions on gums and elevated monocyte and leukocyte counts. EKG changes were seen on day 10 of recovery. At necropsy, lesions were seen on heart, liver, gall bladder and gums. The female had diarrhea, emesis and weight loss. A small spot was found on the pituitary at necropsy. A heart lesion was found in the right ventricular myocardium and was necrotic. Hepatic midzonal degeneration and thymic atrophy were seen in both dogs.

AY-22,989: rapamycin single and multiple intravenous dose range finding study-dog
Study no. GTR-19208 non glp
18 Oct 1990

Rapamycin was administered by intravenous injection to beagle dogs at doses of 0 (98% PEG 300+2% polysorbate 80 or 93% PEG 300+2% polysorbate 80 +5% dimethylacetimide), 0.5, 1.0, 2.0, 5.0 or 10.0 mg/kg for one to seven days. One 1.0 mg/kg male was found dead on day 7 of dosing and one female, 10.0 mg/kg, was sacrificed in extremis on day 9 after 7 days of dosing. Remaining dogs were sacrificed 13-16 days after the last day of dosing. All treated dogs had weight loss and decreased food consumption, diarrhea, emesis, blood in feces and decreased activity. At necropsy, one dog (died day 7, 1.0 mg/kg) had necrotizing typhlitis, gastric ulcers, an ileal intussusception and bronchopneumonia with aspiration of emesis. The sacrificed dog (10.0 mg/kg) had gastric ulceration and lymphoid necrosis in the intestine. In the surviving dogs, one splenic infarction (5 mg/kg)and adrenal cortical hypertrophy (10 mg/kg) were seen at necropsy. A NOAEL was not seen in this study.

AY-22,989: rapamycin twenty-eight day intravaginal toxicity study- dog
Study no. GTR-T-75-9 non glp
15 Dec 1975

Female beagle dogs (4/group) were treated with rapamune by intravaginal dosing daily for 28 days, receiving either 0 (cream base) or 200 mg/dog, with doses ranging from 12.98 to 37.03 mg/kg. One drug-treated dog died on day 20 and one was sacrificed in extremis on day 25. All other dogs survived until necropsy. During the study, drug-treated dogs displayed decreased body weight, food consumption and motor activity, mammary gland enlargement, ptosis, salivation and ulceration of the oral mucosa, dehydration, dry skin, loss of hair and mucus exudate of the eyes. Electrocardiograms of the treated group showed a number of abnormalities including ectopic locus of polarization, second degree nodal block and premature ventricular contractions in one dog which had vacuolation of the myocardial cells. A second treated dog had S-T depression, A-V nodal escape, notched QRS, and extra P wave: these were accompanied by acute inflammation and thrombosis of the aorta. A third treatment group dog had S-T depression, second degree nodal block, premature ventricular contractions and ectopic atrial depolarization. Histopathologic findings included an area of myocardial infarction with areas of diffuse ischemic necrosis and inflammatory cell infiltration. Focal necrosis, monocyte infiltration and thrombosis were seen in the aorta. The fourth treatment dog had a depressed S-T segment and premature ventricular contractions, but no cardiovascular histopathologic findings. RBC count, hematocrit and hemoglobin were decreased in treated dogs. Cholesterol and creatine kinase were elevated in all treated dogs. At necropsy adrenal weights were increased in the treated group. In addition to the cardiovascular histopathologic changes noted above, marked lymphoid and thymic atrophy were seen in the treated dogs.

AY-22,989: rapamycin four week vaginal toxicity study- dog
Study no. GTR-T-75-30 non glp
18 Nov 1975

Female beagle dogs (4/group) were treated with rapamune by intravaginal dosing daily for 28 days, receiving 0 (cream base) 20, 60 or 200 mg/dog, with actual doses of 3.20, 11.80 and 37.26 mg/kg, respectively. During the study, mucoid red or green diarrhea was seen in dogs receiving 20 mg; one died on day 14. Two drug-treated dogs receiving 60 mg died during the study (days 25 and 26) and one dog receiving 200 mg was sacrificed in extremis on day 14. All other dogs survived until necropsy. Other treated dogs had ulceration of the oral mucosa, decreased body weight and food consumption, increased cholesterol and leukocyte counts and decreased chloride. Increased urea nitrogen was seen in dogs receiving 60 and 200 mg. Slight amounts of albumin was seen in urine of treated dogs as well as occult blood in urine of dogs receiving 200 mg. At necropsy, hypoplasia of the vaginal epithelium was seen in treated dogs as well as ulceration and inflammation of the oral and gastrointestinal epithelium. Myocardial necrosis, vasculitis, regenerative kidney tubules and casts were seen in the 60 and 200 mg groups. All treated dogs had generalized wasting with hypoplasia of the lymph nodes, spleen, bone marrow, skeletal muscle atrophy, thymic involution and degranulation of pancreatic acinar epithelium. A NOAEL was not achieved in this study.

AY-22,989 preliminary tolerance toxicity study- cynomologus monkey
study no. GTR -17270
23 May 1989

One male and one female cynomologus monkey [redacted] were administered AY-22,989 by oral intubation as a daily dose of 15 mg/kg/day for three days. Observations continued for three days after the last dose. No effects were seen.

AY-22,989:one month oral (gavage) toxicity study-cynomologus monkey
Study no. GTR-17195 non glp
12 May 1989

Cynomologus monkeys [redacted] 2/sex/dose) were administered rapamycin daily by oral gavage for four weeks at doses of 0 (1% carboxymethyl cellulose), 5, 10 or 15 mg/kg. All animals survived until necropsy. During the study, no drug-related signs were seen in the treated groups; however, the small group sizes used in this study precludes treatment relationships to any non-overt signs. At necropsy, no overt treatment related signs were seen.

AY-22,989 rapamycin: intravenous dose range finding study-monkey
Study no. GTR-19212 non glp
26 Oct 1990

Cynomologous monkeys [redacted] were administered rapamycin by daily intravenous injection. The animals were treated according to the following dose schedule:

Dose mg/kg	vehicle	♂	♀	Days of treatment	Days of observation post-dosing
0	A	6		7	13
0	B		8	7	14
0	C	11		7	14
0.5	A	4		7	4
0.5	A	9		7	14
0.5	A	10		7	14
0.5	D		12	22	6
0.5	D		13	22	6
1.0	D		14	20	6
1.0	A	2		8	13
2.0	A	1		7	14
2.5	D	15		20	6
5.0	A		3	5	16
5.0	D	16		13	6
10.0	A		5	7	14
10.0	A		7	10	14

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Vehicles
A= PEG 300, polysorbate 80
B= PEG 300, polysorbate 80, dimethylacetimide
C= Polysorbate 80, water
D= PEG 300, polysorbate 80, dimethylacetimide and water

All monkeys survived until necropsy. During the study, slight body weight decreases occurred in drug-treated monkeys after one week. No other overt drug-related signs were seen during the study. Necropsies were not preformed.

AY-22,989 rapamycin:one month intravenous toxicity study-monkey
Study no. GTR-20212
4 Sept 1991

Cynomologous monkeys [redacted] (3 male/2 female/dose) were administered rapamycin by daily intravenous injection for twenty-eight days. Doses of 0 (PEG 300, polysorbate 80, dimethylacetimide and water), 0.025 and 0.1 mg/kg were used. All monkeys survived until necropsy. During the study no drug-related signs were seen. At necropsy decreased RBCs, hemoglobin and hematocrit and lymphoid and thymic atrophy were seen in treated animals. Other than the immunosuppressive effects, no adverse effects were seen at either dose.

AY-22,989 rapamycin:one month intravenous toxicity study-monkey
Study no. GTR-19211
11 Dec 1990

Cynomologous monkeys [redacted] (3 /sex/dose) were administered rapamycin by daily intravenous injection for twenty-eight days. Doses of 0 (PEG 300, polysorbate 80, dimethylacetimide and water), 0.25, 0.75 and 2.50 mg/kg were used. All monkeys survived until necropsy. During the study a dose related body weight decrease (up to 21%) was seen. Food consumption was decreased in drug-treated animals. Injury (swelling, focal lesions) at the injection site in both control and treatment monkeys necessitated interruptions in dosing or partial doses in monkeys of each dose group. Colitis occurred in each

treatment group. At necropsy, lymphoid atrophy (spleen, lymph nodes and thymus) was seen in all drug treated monkeys. Periarterial edema in the heart, liver or gall bladder was seen in the 0.75 mg/kg group, and myocardial hemorrhage in one 2.50 mg/kg animal. The NOAEL for this study was 0.25 mg/kg.

Sirolimus: Three week oral (gavage) dose ranging study in mice (addendum I)
GTR-24957
14 Dec 1994

Rapamune was administered to CD-1 mice (Charles River; 5/sex/group) by oral gavage at doses of 0 (1% polysorbate 80, 99% phosal 50, PG), 5, 10 and 20 mg/kg for three weeks. All mice survived until necropsy. During the study, body weights and food consumption of treated males decreased slightly. Hematology changes (drug related) included increased RBCs, hemaglobin and hematocrit (all groups), decreased platelets (10 and 20 mg/kg males and all females), decreased lymphocytes (all males and 20 mg/kg females), increased neutrophils (all groups), increased monocytes (5 and 10 mg/kg males), and decreased eosinophils (10 and 20 mg/kg males and all females). Drug-related clinical chemistry changes included increased ALT (10 mg/kg males and all females), increased AST (5 and 10 mg/kg males and all females), increased alkaline phosphatase (5 mg/kg males and 5 and 10 mg/kg females), increased cholesterol (all drug-treated groups), increased triglycerides (5 mg/kg males and all females), and increased sodium and decreased chloride and inorganic phosphate (all males). At necropsy, lymphoid atrophy was seen in all treated groups. Testicular tubular degeneration and tubular giant cells were seen in all male groups. A NOAEL was not obtained in this study.

Rapamune: Thirteen Week Oral (Gavage) Ranging Study in B6C3F1 Mice
GTR-30332
3 Feb 1998

Rapamune was administered orally to B6C3F$_1$ mice ([redacted] 10/sex/dose) at doses of 0 (1% polysorbate 80 and 99% phosal 50 PG), 10, 30 or 100 mg/kg for 13 weeks. Additional groups (10 mice/sex group) received rapamune for 13 or 14 days for pharmacokinetic evaluation. During the study, two males (100 mg/kg) and three females (10, 30, 100 mg/kg) died. These deaths were attributed to gavage or cage accidents or purulent pyelonephritis. All other mice survived until necropsy. During the study, slight decreases were seen in body weight and food consumption in drug-treated mice. Skin lesions on the ear and anterior back were seen after day 21 of the study on treated mice. Hematologic changes included increased rbc and decreased eosinophils (all groups). White blood cells, lymphocytes and neutrophils were decreased from controls (37.3 to 52.0%) in the 30 and/or 100 mg/kg male groups and eosinophils were decreased from controls in male and female groups (36.4 to 75%). At necropsy the following organ weight changes were observed: liver, increased in 30 and 100 mg/kg mice; testes, decreased in all treated males; kidneys, decreased in treated females; and brain, decreased in 30 and 100 mg/kg mice. Skin abrasions were seen in 30 and 100 mg/kg mice and small testes in all treated male groups. Histopathology findings included hypospermia and immature spermatozoa in all treated male groups, tubular atrophy of the testes, all treated males, thymic atrophy, all treated groups, and uterine atrophy, all treated female groups. Pharmacokinetic parameters appear below.

MEAN (±SE) TOXICOKINETIC PARAMETERS

DOSAGE	MALE		FEMALE	
(mg/kg)	Cmax (ng/mL)	AUC_{0-8} (ng•hr/mL)	Cmax (ng/mL)	AUC_{0-8} (ng•hr/mL)
10	310 ± 99	1899 ± 326	603 ± 373	1869 ± 564
30	1968 ± 100	12,418 ± 606	2240 ± 804	15,383 ± 2548
100	3444 ± 60	23,922 ± 1282	4192 ± 588	27,900 ± 901

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A NOAEL was not achieved in this study.

Rapamune: special oral (gavage) study to evaluate the reversibility of the effects on testes and bone in young male rats
GTR-26739
20 Mar 1998

Male CD rats [redacted] were dosed with rapamune daily for 13 weeks at doses of 0 (0.1 % polysorbate 80 NF, 9.9% phosal 50 PG), 2 or 6 mg/kg; 40/dose group. Ten rats/dose group were sacrificed at the end of treatment and after recovery periods of 1, 3 or 6 months to determine hormonal and bone effects of rapamune. During the study three rats died or were euthanized. One control rat died of gavage trauma. One control rat was euthanized with hind limb paralysis attributed to CNS lymphosarcoma. A 6 mg/kg rat was euthanized on day 79 with bone fractures and osteomyelitis of the left femur. Other findings during the study included decreased body weight gain in treated rats during treatment. During recovery, weight gain was increased for treated rats but did not recover to control weights. Food consumption was increased in treatment rats during treatment and recovery. Lameness was seen in four rats receiving 6 mg/kg. Testosterone levels were variable during treatment and recovery relative to control levels. Leutinizing hormone and follicle stimulating hormone were increased in 6 mg/kg rats relative to controls during and after treatment. Bone mineral area, femoral bone strength and mineral density, cortical thickness and breaking point were decreased for treated rats and did not recover following treatment. Sperm counts were significantly reduced in 6 mg/kg rats, with partial recovery at 3 and 6 months. Dose dependent reductions in weight of testes, epididymides and prostate were seen in treated rats, with partial recovery through 6 months. In the testes, tubular degeneration and testicular edema were seen at 2 and 6 mg/kg and oligospermia and prostate atrophy in the 6 mg/kg group. Cataracts were also seen in the 6 mg/kg group. Partial reversals of reproductive organ effects were seen after 6 month recovery while recovery from adverse bone effects was minimal.

Rapamune: special oral (gavage) study to evaluate the effects on bone quality and testes in older male rats
GTR-26105
28 Sept 1998

Male CD rats [redacted] 10/age/dose), age 8 weeks and 9 months, were administered rapamune daily by oral gavage at doses of 0 (0.1 % polysorbate 80 NF, 9.9% phosal 50 PG), 2 or 6 mg/kg for 21 days. All rats survived until necropsy. During the study, one 2 mg/kg young rat and two 6 mg/kg young rats exhibited lameness. Group body weights were reduced in young rats receiving 2 or 6 mg/kg and old rats receiving 6 mg/kg. Food consumption was reduced in young rats receiving 6 mg/kg and old rats receiving 2 or 6 mg/kg. At necropsy small testes were seen in young or old rats receiving 2 or 6 mg/kg. Testicular tubular degeneration was seen in young and old rats receiving 6 mg/kg. Testosterone was decreased in both young and old rats receiving rapamune in a dose dependent manner. Bone fractures were found in one young rat receiving 2 mg/kg and two young rats receiving 6 mg/kg. Bone mineral density and length and strength of femurs were reduced in young rapamune-treated rats.

Rapamune: mechanistic study to evaluate the effects of testosterone replacement on bone in rapamune treated male rats
GTR-31388 non glp
24 June 1998

Male CD rats [redacted] were dosed with rapamune at 0 (.1 % polysorbate 80 NF, 9.9% phosal 50 PG), 6 mg/kg or 6 mg/kg and 20 mg testosterone (subcutaneous implant) for 21 days in groups of 10. All rats survived until necropsy. During the study, body weights and food consumption in rapamune treated rats were decreased. With variation, the rapamune treated rats had lower group mean testosterone than controls while testosterone treated rats had higher group mean levels. Rapamune decreased femoral length, bone mineral density, strength, cortical width and bone cortical mineral area compared to controls. Addition of testosterone partially reversed the rapamune-induced bone deficits.

Rapamune: 13 week oral (gavage) phospholipidosis study in male rats with a 4 week recovery
GTR-32615
15 Oct 1998

Male rats [redacted] were given rapamune by oral gavage for 13 weeks at doses of either 0 (0.1% polysorbate 80, 9.9% phosal 50 PG) or 6 mg/kg, 40/group. A four week recovery period followed. Alveolar macrophages were harvested by bronchoalveolar lavage at week 14 and the end of the recovery period. One rat receiving 6 mg/kg rapamune was euthanized on day 85 due to body weight loss. All other rats survived until necropsy. During the study, rats receiving rapamune experienced body weight gain. Food consumption was decreased in treated rats during the initial five weeks of treatment. Lameness (14/40), eye opacity and soft stools were seen in treated rats. Alveolar macrophages taken at week 14 showed increased total phospholipid content (19 fold) in treated rats. Phospholipid content remained elevated after the four week recovery (11 fold). Electron microscopy confirmed the presence of lamellar inclusion bodies in the macrophages, with a reduction following recovery

Toxicity of rapamycin- a comparative and combinative study with cyclosporine at immunotherapeutic dosage in the rat
GTR-20619 non glp manuscript

Sprague Dawley rats were treated for 14 days with rapamycin (1.5 mg/kg, i.p.), cyclosporin (15 mg/kg, p.o.) or both. Rapamycin and combination treated rats had reduced weight gain. Total white cell counts and T and B cells were unaffected by individual drug treatment but leukopenia was seen in the combination. The individual drugs slightly increased urinary flow and markedly in the combination. Focal tubular necrosis was seen in the cyclosporin treated rats but not in rapamycin treated rats. Functional impairment due to cyclosporin was exacerbated by rapamycin. Serum glucose was increased by both drugs and additionally in combination treated rats. Rapamycin and combination treated rats also showed focal myocardial necrosis

Rapamune/cyclosporine: thirteen week oral (gavage) toxicity study in rats
GTR-31710
28 Aug 1998

The combination of rapamune/cyclosporin was administered by oral gavage to male and female rats [redacted] 20/sex/dose) for 13 weeks at doses of 0/0 (0.1% polysorbate 80, 9.9% phosal 50 PG, 0.038% dehydrated alcohol), 0.5/0, 0/2 or 0.5/2 mg/kg. All rats survived until necropsy. During the study, males of groups treated with rapamune had reduced body weight gains, and the group receiving the rapamune/cyclosporin combination had the most reduced body weight gain. Food consumption was also decreased in males. Females were unaffected. Major hematologic effects of rapamune included increased neutrophils, decreased lymphocytes and WBCs (females) and decreased eosinophils. Clinical chemistry findings included evidence of interaction of the combination (increases in ALT, alkaline phosphatase and potassium and decreases in total bilirubin). Lymphoid atrophy was seen in all treated groups; myocardial degeneration (rapamune related) and increased incidence of pulmonary alveolar macrophages (especially in the combination group) were seen at necropsy.

Rapamycin: seven day intravenous irritation study in rabbits
GTR-23138 non glp
15 November 1993

Male New Zealand White rabbits [redacted] were given i.v. injection of rapamycin at doses of 0 (16.6 % propylene glycol, 21.5% polyethylene glycol 400, 1% polysorbate 80, 60.9% water, 0.002 %EDTA) or 1 ml/day, 1 mg/ml for 7 days. All rabbits survived the study. Both vehicle and drug groups had intravenous irritation.

Sirolimus: DOT acute dermal irritation study on rabbits
GTR-25355 non glp
18 November 1994

Rapamycin was administered in a single dermal application to male and female New Zealand White rabbits [redacted].A dose of 200 mg/kg was applies with white petrolatum on cotton patches on the backs of rabbits and observed for 48 hr. No overt clinical signs were seen.

REPRODUCTIVE TOXICOLOGY STUDIES
Fertility and Reproductive Performance
Rat
Rapamycin: Rat Fertility Dose Range Study – Oral Intubation GTR-22664

Rapamycin: Study of Fertility and General Reproductive Performance With "Behavioral" and Reproductive Assessment of The Offspring (Segment I) - Rat - Oral Intubation GTR-22358

Rapamycin: Oral (Gavage) Fertility and General Reproductive Performance (Segment I) Study in Rats GTR-23840

Developmental Toxicity
Rat
Rapamycin: Rat Developmental Toxicity Dose Range Study - Oral Intubation GTR-21936 (attached)

Rapamycin: Developmental Toxicity Study (Segment II) - Rat - Oral Intubation GTR-22355 (attached)

Rapamune/Cyclosporine: Oral (Gavage) Developmental Toxicity Study in Rats GTR-31140

Rabbit
Rapamycin: Single Dose Oral (Gavage) Range Finding Study - Nongravid Female Rabbit GTR-22571 (attached)

Rapamycin: Rabbit Developmental Toxicity Dose Range Study - Oral Intubation GTR-22572 (attached)

Rapamycin: Developmental Toxicity Study (Segment II) - Rabbit - Oral Intubation GTR-22357 (attached)

Perinatal - Postnatal Toxicity
Rat
Sirolimus: Oral (Gavage) Perinatal and Postnatal with Behavioral and Reproductive Assessment of the Offspring (Segment III) Study in Rats GTR-24874 (attached)

REPRODUCTIVE TOXICOLOGY STUDY REVIEW

Rapamycin: rat fertility dose range study-oral intubation
GTR-22664
23 Apr 1993

Male and female rats [redacted](CD; 2 subgroups, 8/sex/dose) were assigned to two subgroups, one with males receiving treatment (daily oral gavage, four weeks prior to cohabitation with untreated females, continuing until necropsy) and another subgroup with females receiving treatment (daily oral gavage two weeks prior to cohabitation with untreated males and continuing until necropsy). Doses of 0 (0.2% dimethylacetimide, 0.05% polysorbate 80 NF and 0.75% phosal 50), 0.1, 0.5, 2 and 5 mg/kg were used. All rats survived until necropsy. In the male treatment subgroup, body weight and food consumption were reduced at 2 and 5 mg/kg. At necropsy, atrophic testes and reduced seminal vesicles were seen in males receiving 2 and 5 mg/kg. Untreated females showed no gross or histologic effects. Pregnancy rates were

affected as 2/8 (2 mg/kg male) and 3/8 females (5 mg/kg male) were not pregnant. In the female treatment study, decreased weight and food consumption were seen in the 2 and 5 mg/kg groups. On gestation day 13, caesarian deliveries were performed and increased resorptions and dead embryos were seen at doses of 0.5, 2 and 5 mg/kg as well as increases in number of copora lutea. Total implantations were reduced in the 2 and 5 mg/kg groups. No other drug related effects were seen. The NOAEL for this study was 0.5 mg/kg in males and 0.1 mg/kg in females.

Rapamycin: study of fertility and general reproductive performance with "behavioral" and reproductive assessment of the offspring (segment I) study in rats
GTR-22358
7 Aug 1998

Male and female rats (Charles River CD) were assigned to two subgroups, one with males receiving treatment (24/sex/dose; daily oral gavage, 11 weeks prior to cohabitation with untreated females, continuing until necropsy on gestation day 21). Doses of 0 (0.2% dimethylacetamide, 0.05% polysorbate 80 NF and 0.75% phosal 50), 0.1, 0.5 and 2 mg/kg were used. In another subgroup with females receiving treatment (30/sex/group; daily oral gavage two weeks prior to cohabitation with untreated males and continuing until necropsy on gestation day 21, 15 treated females/group) or postpartum day 21 (15 treated females/group), doses of 0 (0.2% dimethylacetamide, 0.05% polysorbate 80 NF and 0.75% phosal 50), 0.05, 0.1 and 0.5 mg/kg were used. F1 offspring were evaluated and selected for mating with members from the same parental treatment group. All rats survived until necropsy. During the study, treated males had reduced weight gain and food consumption. Reduced fertility was seen in the 2 mg/kg group. At necropsy, the 2 mg/kg group had reduced reproductive organ weights, focal testicular mineralization, slight to severe tubular atrophy, slight giant cell formation and mild to severe hypospermia of the testes and moderate to severe hypospermia of the epididymes. No effects were seen in the F1 generation. All treated females survived until necropsy. Gravid uterine weights were decreased in the 0.5 mg/kg group as well as an increase in resorptions, reduced litter size and fewer live pups. No effects were seen in the F1 generation or F2 generation. The NOAEL for this study was 0.5 mg/kg for males, 0.1 mg/kg for females, with fetal toxicity observed at a maternal dose of 0.5 mg/kg.

Rapamycin: study of fertility and general reproductive performance with "behavioral" and reproductive assessment of the offspring (segment I) study in rats
GTR-23840
7 Aug 1998

Male and female rats (Charles River CD) were assigned to two subgroups, one with males receiving treatment (24/sex/dose; daily oral gavage, 11 weeks prior to cohabitation with untreated females, continuing until necropsy on gestation day 21). Doses of 0 (0.2% dimethylacetamide, 0.05% polysorbate 80 NF and 0.75% phosal 50), 0.1, 0.5 and 2 mg/kg were used. In another subgroup with females receiving treatment (30/sex/group; daily oral gavage two weeks prior to cohabitation with untreated males and continuing until necropsy on gestation day 21 (15 treated females/group) or postpartum day 21 (15 treated females/group), doses of 0 (0.2% dimethylacetamide, 0.05% polysorbate 80 NF and 0.75% phosal 50), 0.05, 0.1 and 0.5 mg/kg were used. F1 offspring were evaluated and selected for mating with members from the same parental treatment group. All rats survived until necropsy. During the study, treated males had reduced weight gain and food consumption. Reduced fertility was seen in the 2 mg/kg group. At necropsy, the 2 mg/kg group had reduced reproductive organ weights, focal testicular mineralization, slight to severe tubular atrophy, slight giant cell formation and mild to severe hypospermia of the testes and moderate to severe hypospermia of the epididymes. No effects were seen in the F1 generation. All treated females survived until necropsy. Gravid uterine weights were decreased in the 0.5 mg/kg group as well as an increase in resorptions, reduced litter size and fewer live pups. No effects were seen in the F1 generation or F2 generation. The NOAEL for this study was 0.5 mg/kg for males, 0.1 mg/kg for females, with fetal toxicity at a maternal dose of 0.5 mg/kg.

Rapamune/cyclosporine: oral (gavage) developmental toxicity study in rats
GTR-31140
22 July 1993

Time-mated female rats (Charles River CD [redacted] were treated with a combination of rapamune/cyclosporine (Neoral ™, Sandoz Pharma Ltd.) at doses of 0/0 [0.1% polysorbate 80, 9.9% phosal 50 PG and water (rapamune) and 0.038% dehydrated alcohol in water (Neoral)], 0.5/0, 0/2 and 0.5/2 mg/kg from day 6 of gestation until necropsy on gestation day 21. All rats survived until necropsy. During the study, salivation was seen in rats treated with cyclosporine. At necropsy, the combination group had significantly greater embryo and fetal mortality than rapamune alone. Body weight gain on gestation days 13 to 20 was also significantly reduced, as was gravid uterine weight and mean number of live fetuses. A statistically significant fetal effect was seen in the cyclosporine and rapamune/cyclosporine groups: increased incidence of cervical ribs. Fetal mortality was increased by the rapamune/cyclosporine combination.

GENETIC TOXICOLOGY STUDIES

In Vitro Non-mammalian Cell System Evaluation of AY-22989 in the *Salmonella*/Microsome Mutagenicity Test (Ames Assay) GTR-18811

In Vitro Mammalian Cell System Mutagenicity Test on Rapamycin in the L5178Y TK+/- Mouse Lymphoma Forward Mutation Assay with a Confirmatory Assay GTR-25495 (attached)

Mutagenicity Test on Rapamycin: Chromosomal Aberrations in Chinese Hamster Ovary (CHO) Cells with a Confirmatory Assay with Multiple Harvests GTR-22683 (attached)

In Vivo Mammalian System Mutagenicity Test on Rapamycin in an In Vivo Mouse Micronucleus Assay GTR-25481 (attached)

GENETIC TOXICOLOGY STUDY REVIEW

Evaluation of AY-22,989 in the *Salmonella*/microsome mutagenicity test (Ames assay)
Study no. GTR-18811
1 Aug 1990

AY-22,989 was tested in the Ames mutagenicity assay using *Salmonella* strains TA97, TA98, TA100, TA102 and TA104, with and without metabolic activation (Araclor 1254-induced rat S-9 liver fraction). Doses of AY-22,989 used were 0.5, 5, 50, 500 and 5000 µg/plate in 0.1 ml. Drug was dissolved in DMSO. Positive controls included ICR-191, sodium azide, cumene hydroperoxide, methylglyoxal, and 2-amino-fluorene and were effective. No increase in revertants was seen due to drug exposure. AY-22,989 appears to be non-mutagenic within the range of the concentrations tested.

NONCLINICAL PHARMACOKINETIC STUDIES

Drug Metabolism - Study Reports
Structure of Labeled Compound
Rat
Recovery of Radioactivity in the Expired Air, Urine and Feces of Rats Receiving a Single Oral Dose of [14C] Rapamycin GTR-20974

Mouse
Single-Dose Study
Rapamune: Single Oral (Gavage) Dose Toxicokinetic Study in Male Mice: Bioanalytical Report (Protocol 97117) GTR-34132

Repeated Dose Studies
Rapamune(TM): Six Week Oral (Gavage) Ranging Study in B6C3F1 Mice: Whole Blood Concentrations at 14 Days (Protocol 96062) GTR-29912

Sirolimus: Thirteen Week Oral (Gavage) Toxicity Study in Mice - Bioanalytical Results (Protocol 94061) GTR-25695

Sirolimus: Thirteen Week Oral (Gavage) Toxicity Study in Mice - Bioanalytical Results (Protocol 95013) GTR-26272 (attached)

Sirolimus: Single and Multiple (14 Days) Dose Oral (Gavage) Toxicokinetic Study in Male Mice (Protocol 95701) GTR-26455 (attached)

Rapamune(TM) (AY-22989, Rapamycin, Sirolimus):Multiple (14 Days) Dose Oral (Gav) Toxicokinetic Study in B6C3F1 Mice (Protocol 96828) GTR-31075

Rapamune TM: A Twenty-Nine Week Chronic (Male) and Eighty-Six Week Carcinogenicity (Female) Oral (Gavage) Study in Mice: Whole Blood Sirolimus
Concentrations (Protocol 95104) GTR-33690

AY-22989/Rapamycin/Sirolimus/Rapamune(TM): Two Year Oral (Gavage) Carcinogenicity Study in Mice-II: Whole Blood Concentrations and
Pharmacokinetics at 52 Weeks (Protocol 96047) GTR-33372

Rat
Single Dose Studies
Pharmacokinetics of Rapamycin (AY-22989) After Ascending Intravenous Doses in Rats GTR-19405 (attached)

The Concentrations of Rapamycin (AY-22989) in Cynomolgus Monkeys and Rats Following Single Oral Doses of Rapamycin GTR-20232 (attached)

Pharmacokinetics of Rapamycin and Three of Its Primary Metabolites in Rats After an Oral 9.5 mg/kg Dose of Rapamycin GTR-23305

The Bioavailability and Pharmacokinetics of Rapamycin (AY-22989) in Rats Receiving a Single 0.25 mg/kg IV Dose of Rapamycin GTR-20884 (attached)

Presystemic Extraction of Rapamycin in Rats GTR-20540 (attached)

Factors Affecting the Oral Bioavailability of Rapamycin and Its Metabolites in the Rat GTR-21154 (attached)

Rapamycin: Pharmacokinetics After Single IG Doses in Rats GTR-22748 (attached)

The Relative Bioavailability of Rapamycin From Non-DMA Versus DMA Containing Oral Formulations in Rats GTR-22327 (attached)

Repeated-Dose Studies Pharmacokinetics of Rapamycin (AY-22989) in Rats
Following IV Doses of Rapamycin (1.5 mg/kg/day) for One or Seven Days GTR-18908 (attached)

Accumulation of Radioactivity in the Blood of Rats Receiving [3H]-Rapamycin IG for Seven Consecutive Days (0.8 mg/kg/day) GTR-20664 (attached)

Rapamycin: Pharmacokinetics After Single and Multiple (14 Days) IG Dosing (0.25 mg/kg/day) in Rats GTR-23003 (attached)

Rapamycin: Single and Multiple (14 Days) Dose Oral (Gavage) Pharmacokinetic Study Following Doses of 0.2, 2.0, and 6.0 mg/kg in Rats GTR-24366 (attached)

Rapamune(TM) and WAY-126792 (Seco-Rapamycin): Twenty-Eight Day Oral (Gavage) Toxicity Study in Male Rats (Protocol 95102) GTR-27897

Sirolimus and WAY-124854 (Sirolimus Impurity): Twenty-Eight Day Oral (Gavage) Toxicity Study in Male Rats (Protocol 95095) GTR-27116

Sirolimus: Fifty-Two Week Oral (Gavage) Toxicity Study in Rats - Bioanalytical Results (Protocol 93021) GTR-25696

Rapamune(TM): Multiple Dose (10 Days) Oral (Gavage) Toxicokinetic Study in Gravid Rats: Pharmacokinetic Data (Protocol 97036) GTR-33239

Rapamune(TM): Two Year Oral (Gavage) Carcinogenicity Study in Rats: Whole Blood Sirolimus Concentrations (Protocol 95103) GTR-33302

Rabbit
Single Dose Studies
The Pharmacokinetics of Rapamycin After Single Intravenous Doses to Rabbits GTR-22119

Multiple Dose Studies
Rapamune[TM]: Multiple Dose (13 Days) Oral
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Method for the Quantitation of Rapamycin (AY-22989) in Human Whole Blood and the Plasma and Whole Blood of Rats and Cynomolgus Monkeys- Mann Testing Assay GTR-22749 (attached)

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Rapamune[TM]: An Method for the Quantitation of Rapamune in Rat Whole Blood [Range 0.5 To 100 ng/mL] (Protocol 96991) GTR-32861

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Sirolimus (AY-22989, Rapamycin, Rapamune(TM)): Stability and additional validation data for the quantification of sirolimus in human whole blood and plasma using [redacted] (Protocols: DM931093, 95774, DM941018, 95775, 96766, 96964) GTR-33371

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Pharmacokinetic Interaction Between Rapamycin and Cyclosporine When Administered Orally to Rats GTR-22280 (attached)

Sirolimus (Rapamycin): Inhibition of CYP3A Catalyzed Demethylation and Hydroxylation by Cyclosporine in Human Liver Microsomes - Estimate of In Vivo Drug Interaction GTR-32184

Rapamycin: The Effect of Normal and High Fat Food on the Relative Bioavailability in Cynomolgus Monkeys Following Oral (Intragastric) Administration (0.2 mg/kg) GTR-24310 (attached)

Rapamycin: Conversion of Isomer C to Isomer B in the Rat Following Single Intragastric (5 mg/kg) and Intravenous (1 mg/kg) Administration of Isomer C GTR-24942 (attached)

Rapamycin: Effects on Testicular Steroidogenic Enzymes GTR-27894 (attached)

Rapamune(TM): 13 Week Oral (Gavage) Phospholipidosis Study in Male Rats With a 4 Week Recovery: Bioanalytical Report (Protocol 96248) GTR-33812

NONCLINICAL PHARMACOKINETIC REPORTS

Recovery of radioactivity in the expired air, urine and feces of rats receiving a single dose of [14C] rapamycin
GTR-20974 non glp
23 March 1992

Two Sprague Dawley rats (Charles River) received a 5.0 mg/kg i.g. dose of [14C]rapamycin (lot no. CFQ 6485, specific activity 12.8 μCi/mg) with an individual dose of 45μCi/kg. The 24 hr recovery of radioactivity in the expired air was less than 0.05% of the administered dose. After 7 days, the total recovery of radioactivity was 97.1%, much greater than the recovery in a previous study using [3H]rapamycin (GTR 20239). Of the recovered radioactivity, 97.4% was found in the feces and 2.6% was found in the urine. The feces was the primary route of radiolabel excretion.

Rapamune single oral (gavage) dose toxicokinetic study in male mice: bioanalytical report (protocol 97117)
GTR-34132
31 August 1998

Rapamycin was administered in a single oral (gavage) dose to male CD-1 mice at a dose of 5000 mg/kg and drug concentrations in whole blood were determined by an [redacted] method. The rapamycin concentrations measured at 2, 4 and 8 hours were similar; the highest mean (± SE) concentration (Cmax)

was 3.03 ± 0.2 μg/mL and occurred at 8 hours post-dose. The AUC0-∞ was 49.8 ± 2.8 mg·hr/mL and the apparent terminal half-life was 6.6 ± 0.8 hours.

Rapamune: six week oral (gavage) ranging study in B6C3F1 mice: whole blood concentrations at 14 days (protocol 96062)
GTR-29912
12 December 1997

Male and female mice (2/sex/timeoint) were administered single oral doses of 10, 30, and 100 mg/kg of rapamycin daily for 14 days. Whole blood concentrations of rapamycin were determined by a validated HPLC method. Mean rapamycin Cmax (± SE) values after 10, 30, and 100 mg/kg of Rapamune were 310 ± 99, 1968 ± 100 and 3444 ± 60 ng/mL, in males, and 603 ± 373, 2240 ± 804, and 4192 ± 588 ng/mL, in females, respectively. Corresponding rapamycin AUC values were 1899 ± 326, 12418 ± 606, and 23922 ± 1282 nghr/mL (males) and 1869 ± 564, 15383 ± 2548, and 27900 ± 901 nghr/mL (females), respectively. With the exception of the AUC0-8 values in the 100 mg/kg/day dose groups, there were no significant differences in the exposure to rapamycin between male and female mice. The data were insufficient to fully assess dose proportionality.

Sirolimus; thirteen week oral (gavage) toxicity study in mice-bioanalytical results (protocol 94061)
GTR-25695
13 February 1995

Rapamycin was administered to male and female CD-1 mice at daily doses of 1, 2.5, 5, 10 and 20 mg/kg in a 3 week oral (gavage) toxicity study. Whole blood samples were collected on the last study day approximately 2 hr after drug administration. Following daily rapamycin doses of 1, 2.5, 5, 10 and 20 mg/kg for 13 weeks, the mean male 2 hr rapamycin concentration values were 76 ± 40, 268 ± 237, 1316 ± 617, 1358 ± 421 and 2650 ± 422 ng/ml, respectively. The mean female 2 hr rapamycin concentration values were 116 ± 96, 580 ± 106, 1079 ± 623, 1962 ± 1231and 2756 ± 1025 ng/ml, respectively. There were no statistically significant gender differences.

Rapamune (AY-22989, rapamycin, sirolimus): multiple (14 days) dose oral (gav) toxicokinetic study in B6C3F1 mice (protocol 96828)
GTR-31075 non glp
28 October 1998

Rapamycin was administered orally by gavage to male and female B6C3F1 mice (4 sex/timepoint) daily for 14 days at doses of 10, 30, or 100 mg/kg/day. Mean rapamycin Cmax (± SE) values after 10, 30, and 100 mg/kg of rapamycin were 0.26 ± 0.04 , 1.76 ± 0.14, and 3.41 ± 0.21 mg/mL, in males, and 0.48 ± 0.04, 2.44 ± 0.32, and 3.61 ± 0.09 mg/mL, in females, respectively. Corresponding rapamycin AUC 0-24 values were 2.11 ± 0.32 , 19.3 ± 1.2, and 44.6 ± 2.1 mg·hr/mL (males) and 3.50 ± 0.23 , 23.5 ± 2.2, and 48.7 ± 3.0 mg·hr/mL (females), respectively. Both parameters were consistently higher in the female mice, and were significantly different from those in the male animals.

AY-22989/rapamycin/sirulimus/rapamune: two year oral (gavage) carcinogenicity study in mice-II: whole blood concentrations and pharmacokinetics at 52 weeks (protocol 96047)
GTR-33372
30 July 1998

Male and female mice were administered oral (gavage) dosages of 1, 3, and 6 mg/kg of rapamycin once daily for 52 weeks. To assess exposure, blood samples were drawn at pre-dose and at 1, 2, 4, 8, 12, and 24 hours post-dose. Whole blood concentrations of rapamycin were determined by a validated [redacted] method. Mean rapamycin Cmax values after 1, 3, and 6 mg/kg of rapamycin were 214, 472 and 1803 ng/mL, in males, and 315, 1305, and 3530 ng/mL, in females, respectively. Corresponding rapamycin AUC0-24 values (± SE) were 797 ± 298, 2018 ± 540, and 6162 ± 785 nghr/mL (males) and 951 ± 335, 5814± 2037, and

10174 ± 2458 ng.hr/mL (females), respectively. Due to the small sample size (n = 2/time point/group) and the variability in the data, no statistical comparisons were made to assess gender differences or dose proportionality.

Pharmacokinetics of rapamycin and three of its primary metabolites in rats after an oral 9.5 mg/kg dose of rapamycin
GTR-23305 non glp
8 December 1993

Rats received a single 9.5 mg/kg oral dose of rapamycin and plasma was collected at 0, 0.5, 1, 2, 4, 7 and 24 hr post-dosing. Plasma concentrations of rapamycin and its metabolites M2 (WAY-130563; an oxygenated metabolite), M3 (WAY- 126792; a ring-opened degradation product) and M5 (WAY-130562; an O-demethylated metabolite) were determined by [redacted]. The drug was rapidly absorbed with tmax in plasma at 0.5 hr after dosing (Cmax = 361 ng/ml). The Cmax and tmax of M2, M3 and M5 were 113 ng/ml and 0.5 hr, 46 ng/ml and 1.0 hr, and 62 ng/ml and 1.0 hr, respectively. The AUCO-∞ of rapamycin, M2, M3 and M5 were 1325, 245, 241 and 227 nghr/ml, respectively. The individual immunosuppressive activities of M2, M3 and M5 by the [redacted] assay were ≤5% of that of rapamycin. Together, the individual AUCo-∞ values of M2, M3 and M5 were <19% of rapamycin.

Rapamune and WAY-126792 (seco-rapamycin): twenty-eight day oral (gavage) toxicity study in male rats (protocol 95102)
GTR-27897
2 July 1996

Male rats were dosed with rapamune, rapamune containing ~5% WAY–126792 (seco-rapamycin) or rapamune containing ~12% WAY–126792 (seco-rapamycin) by oral gavage for 28 days. Doses of 0 (vehicle), 0.1, 1 or 5 mg/kg were given to dose groups of 15 rats. Blood samples were collected from 5 rats/group 1 and 24 hours after the final dose. Whole blood concentrations of rapamune were not affected by the amount of seco-rapamycin co-administered with rapamune.

Sirolimus and WAY-124854 (Sirolimus impurity): twenty-eight day oral (gavage) toxicity study in male rats (protocol 95095)
GTR-27116
21 Mar 1996

Male CD rats were dosed with rapamune, rapamune containing ~3% WAY–124854 or rapamune containing ~10% WAY–124854 by oral gavage for 28 days. Doses of 0 (vehicle), 0.1, 1 or 5 mg/kg were given to dose groups of 15 rats. Blood samples were collected from 5 rats/group 1 and 24 hours after the final dose. Whole blood concentrations of rapamune were decreased by the addition of 10% WAY–124854 co-administered with rapamune.

Sirolimus: fifty-two week oral (gavage) toxicity study in rats -bioanalytical results (protocol 93201)
GTR-25696
5 June 1995

Male and female Sprague Dawley rats in a 52 week oral gavage study had blood sampled at 3 and 50 weeks for Rapamycin determination and calculation of pharmacokinetic parameters (below)

dose mg/kg	AUC male ng.hr/ml	AUC female ng.hr/ml
0.2	17.9	7.9
0.65	40.1	25.1
2	101	71.5
6	430	304

Rapamune: multiple dose (10 days) oral (gavage) toxicokinetic study in gravid rats: pharmacokinetic data (protocol 97036)
GTR-33239
24 June 1998

Rapamycin was administered daily by oral gavage to gravid rats (Charles River CD), for 10 days [gestation days (GD) 6 through 15]. Blood samples were collected in a staggered design on GD 15 and 16 at 1, 2, 4, 8, 10, and 24 hours following GD 15 dosing (at doses of 0.1 and 0.5 mg/kg/day). At 10 days measurable whole blood concentrations were found in gravid rats. Whole blood concentrations of the drug were still measurable 24 hours post-dosing. The mean (± SE) Cmax values for rapamycin after 0.1 and 0.5 mg/kg/day were 0.380 ± 0.028 and 2.57 ± 0.25 ng/mL, respectively and occurred 1 hour post-dose. The corresponding mean AUC0-24 values were 3.42 ± 0.27 and 16.5 ± 1.5 nghr/mL, respectively.

Rapamune: two year oral (gavage) carcinogenicity study in rats: whole blood sirolimus concentrations (protocol 95103)
GTR-33302
24 June 1998

Rapamycin was administered orally by gavage to male and female rats (Charles River CD) for 52 weeks. The dosages used were 0.05, 0.1 and 0.2 mg/kg/day. Blood samples were collected during week 52 at approximately 2 hours after drug administration. The mean (± SD) rapamycin whole blood concentrations at 2 hours post-dose were 0.297 ± 0.162, 0.489 ± 0.320 and 0.678 ± 0.331 ng/mL in male rats administered 0.05, 0.1 or 0.2 mg/kg/day for at least 51 weeks, respectively. In female rats, the corresponding whole blood concentrations were 0.275 ± 0.134, 0.496 ± 0.227 and 0.868 ± 0.345 ng/mL, respectively.

The pharmacokinetics of rapamycin after single intravenous doses to rabbits
GTR-22119 non glp manuscript
28 October 1992

Rapamycin was administered intravenously to five New Zealand White rabbits as single 0.05 or 0.5 mg/kg doses in a crossover fashion and rapamycin concentrations were determined in whole blood by [redacted] up to 24 hr after dosing. Blood levels were quantifiable at 24 hr after each dose (approximately 20 and 60 ng/ml, respectively). Dose-adjusted levels were similar to those found in cynomolgus monkeys but higher than those in rats. Observed clearance values, 0.13 ± 0.01 and 0.06± 0.02 L/kg/hr, respectively, were similar to those seen in monkeys (0.14 ± 0.03 L/kg/hr) but lower than that observed in rats (0.25 L/kg/hr) receiving 0.25 mg/kg iv doses. The whole blood levels of rapamycin after increasing iv doses have not yet been determined in rats or cynomolgus monkeys. However, when measured in the serum of monkeys, both clearance and volume of distribution decreased between iv doses of 0.75 and 2.5 mg/kg. This difference may be due to species difference, or differences in dose. The terminal half lives after 0.05 and 0.5 mg/kg doses (12.8 & 2.1 and 15.3 & 1.2 hr, respectively) are similar to those in monkeys (14.3 hr) but shorter than that in rats (30.8 hr).

Rapamune:multiple dose (13 days) oral (gavage) toxicokinetic study in gravid rabbits: pharmacokinetic data (protocol 97035)
GTR-33259
24 June 1998

Rapamune was administered to New Zealand White SPF rabbits by oral gavage daily on gestation days 6 to 18 at a dose of 0.025 mg/kg. Blood samples were collected at 2, 4, 6, 8, 12 and 24 h post dosing on gestation day 18 for pharmacokinetic analysis. The following pharmacokinetic parameters were determined:

	C_{max} (ng/mL)	t_{max} (hr)	AUC_{0-24} (ng•hr/mL)	$t_{1/2}$ (hr)
Mean	3.40	4.5	45.8	10.5
SD	0.54	1.0	9.3	1.4
SE	0.27	0.5	4.6	0.7
n	4	4	4	4

Sirolimus: 28-day IV study in monkeys (new formulation) (addendum 1) (protocol 94052):bioanalytical results following 23 daily doses
GTR-24928 non glp
26 September 1994

Rapamycin was administered to cynomolgus monkeys at daily doses of 0.01 and 0.025 mg/kg/day in a 28 day intravenous toxicity study. Whole blood samples were collected at 1 hr post-dose on Day 23. Following the 0.01 mg/kg dose of rapamycin, the mean concentrations were 21±5 ng/mL. Following the 0.025 mg/kg dose, the mean concentrations were 40±6 ng/mL.

The distribution of radiolabeled rapamycin within human whole blood
GTR-22348 non glp manuscript
14 January 1993

[3H] Rapamycin and rapamycin were added to EDTA-treated human whole blood to achieve a final radioactivity concentration of 25,000 dpm/ml and total drug concentrations of 5, 10, 25, 50 and 100 mg/ml. The blood was incubated at 37°C for 60 min at which time the formed blood elements (FBEs) and plasma were separated by centrifugation. [3H] Rapamycin was also added to plasma (25,000 dpm/ml, 5-100 ng/ml). The plasma to FBE and plasma to whole blood ratios were 0.05 ± 0.05 and 0.09 ± 0.02, respectively. These values compare to FBE/plasma and whole blood/plasma ratios of 20 and 11, respectively, which correspond to values previously found for human blood in vitro (24 and 12, respectively). Also in agreement with earlier data, there was no temperature or concentration dependence in these ratios in the examined concentration range. The distribution of radioactivity among the blood components was: erythrocytes 94.5 ± 4.9%, plasma 3.1 ± 2.5%,lymphocytes 1.0 ± 1.0%and granulocytes 1.0 ± 0.9%. In the plasma, 2.5 ± 0.2% of the radioactivity was unbound, 19.5 ± 3.9% was bound to HDL, 20.9 ± 5.9% was bound to LDL and 1.2 ± 0.5% was bound to VLDL. The remaining material (about 60% of plasma radioactivity) was assumed to be bound to plasma proteins. Thus, in human blood the great majority of rapamycin is bound to erythrocytes; in plasma, most is bound to proteins or lipoproteins. The amount of free drug in plasma is very low and the unbound fraction of the drug in whole blood is about 0.0870.

Sirolimus (rapamycin): efflux from erythrocytes to plasma in rats and humans
GTR-33255
13 May 1998

In this study, the rates of rapamycin efflux from erythrocytes to plasma at different temperatures were determined in rats and humans using the radiolabeled drug. B/P ratios during the uptake phase were determined at 37C and at a drug concentration of 100 ng/mL. To study drug efflux from erythrocytes, drug-free plasma was used to replace the [14C] drug-containing plasma separated following the uptake phase of the study. Radioactivity concentrations in blood and plasma were determined immediately (time zero) following whole blood reconstitutions, and at different time points for up to 2 hours during the drug efflux phase at 4°C, 22°C and 37°C. In humans, B/P ratios of radioactivity obtained during the uptake phase of this study (average B/P ratio of 13) are consistent with in vitro values reported previously. B/P ratios at time zero of drug efflux in humans are markedly higher (average B/P ratio of 53), and are similar to in vivo values reported in renal transplant patients. In rats, B/P ratios of radioactivity obtained at time zero during the efflux phase (average B/P of 1.8) is also larger than the value determined during the uptake phase (average B/P of 1.1). In both humans and rats, the efflux of radioactivity from erythrocytes to plasma was slow and temperature-dependent, with a re-equilibration time